V.—The Microscopic Sexual Characteristics of the American, Portuguese, and Common Edible Oyster of Europe compared. By John A. Ryder *.

In the issue of 'Forest and Stream' of November 30 just past, in an article by the writer, page 351, middle column, it is remarked:—"I regard Davaine's observations upon the histology of the reproductive organs [of the European oyster] as of little value, being made before the introduction of improved methods of investigation. His figures of the finer structural details have apparently been made from crushed fragments." In passing this judgment upon Dr. Davaine's work I have been severer than the state of the case demanded, as will be seen in the sequel, though I do not yet admit that his methods of research were what they should have been, for until now we have had no adequate description of the structures in question. Until recently I have maintained with reservations that the sexes in the European oyster were probably separate, as in the American; more recent investigation with more refined methods have proved to me that in this I was in error. In my article in 'Forest and Stream' I also took occasion to refer to a statement in Gegenbaur's 'Elements of Comparative Anatomy,' English edition, p. 380, where he says:—"In the oysters we find an intermediate step toward a separation of the sexes, inasmuch as these organs are not active at the same time in the same individual; but the male and female organs alternately so." The writer, in commenting upon the above, then wrote, "This quotation tacitly admits the unisexuality of the European oyster, to which it evidently refers. The last part of the remark, however, is founded upon the slenderest kind of evidence—in fact, upon no evidence except a surmise, as such an alternate activity of the two parts is improbable [for obvious reasons]; besides, it is not possible to demonstrate such an alternation of sexual activity in the same individual. As every one knows, the soft parts of an oyster cannot be examined without opening the shell, which necessarily makes the needed second observation to confirm this alleged alternation of sexual activity a physical impossibility." I am now in a position to go still further, and to assert that the first part of the quotation from Gegenbaur is also erroneous, because we may find both eggs and spermatozoa in the same folliele at the same time.

^{*} From the 'Bulletin of the United-States Fish Commission,' March 14, 1883, pp. 205-215.

What, then, is the true state of the case? This query we propose answering; but before we set out it will be necessary to give some account of the methods of investigation used in order to arrive at a definite conclusion. Thin sections of those portions of the animal in which the reproductive structures are lodged are of the first importance. After trying various methods, which were found for the most part unsatisfactory, the preparation of sections was finally conducted as follows:—After the soft parts were removed from the shell they were thrown into a chromic acid solution of from one to two per cent., in which they were allowed to remain for several days; and in some cases the hardening solution was even renewed. This was done in order that the hardening agent might act upon the whole of the soft parts and harden them throughout; unless the chromic acid is allowed some time to act upon the entire animal, it will not be uniformly hardened, the centre of the body remaining soft. After hardening, the animals should be thoroughly washed and soaked in water for a couple of days, to remove all traces of the acid before they are finally put into alcohol for permanent preservation. Hardened material so preserved will make good sections months afterwards.

Portions of the body-mass of different individuals should then be cut out; it is best to cut up the body into thick slices or blocks in a transverse direction, large enough to be conveniently held between the fingers. It was also found advisable to take such thick slices of the hardened body-mass from several individuals, since it was discovered that scarcely any two had the reproductive glands developed to exactly the same degree of maturity. This point is important, as it has enabled us to follow up the development of the reproductive organs in the connective tissue which invests them. After considerable experiment and disappointment in the effort to imbed these thick hardened slices so as to cut sections with the microtome, the method of imbedding was abandoned altogether. The thick blocks or slices were entirely freed from alcohol by soaking in water for a day, then removed, after drying them off as much as possible with blotting-paper or a soft linen cloth, to a thick solution of gum arabic, in which it is best to allow them to remain from twenty-four to forty-eight hours, so as to be thoroughly saturated. The superfluous gum may then be poured off and the blocks of tissue, soaked as they are with the gum, covered with strong alcohol. In twenty-four hours the blocks will be found hard enough to cut. The blocks of hardened tissue are simply held between the thumb and fore finger, and the sections made with a section-

knife with the free hand. When cutting sections it is necessary to keep the knife well wetted with alcohol, so that the sections may readily slide off on the upperside of the blade. Water should not be used to wet the knife, as it would get on the block of tissue, dissolve the gum, soften the surface to be cut, and injure the succeeding sections. The sections are lifted from the knife as fast as cut, with a camel's hair pencil, and thrown into a dish of water, in which the gum will dissolve out in a few minutes. The sections are then ready to be stained; and in order to clearly differentiate the hermaphroditic character of the reproductive glands of Ostrea edulis, a special staining reagent must be used. The one which gives the best results and acts most quickly will be given here. Equal parts of dense alcoholic solutions of safranin red and methyl green * are poured together and diluted with about eight times their combined volumes of water, producing a dark purplish solution of about the colour of claret wine. Into this the sections may be thrown and allowed to remain until completely saturated with colour, or until they are opaque: they may remain in the staining-fluid from one hour to a day; but two or three hours is a sufficient length of time. When removed from the staining-fluid they are too deeply stained to be mounted at once, and must therefore be transferred to 95per-cent. or absolute alcohol, and stirred about in it until the safranin red is no longer given off in clouds from the sections; but it is important to note that if the sections remain in the strong alcohol too long the whole of the safranin will be washed In order to prevent this, when it is seen that the section has acquired a rosy red hue, combined with a bluish-green tint in the parts stained by the methyl green, the object should at once be removed from the alcohol, thrown into oil of cloves and mounted in balsam or damar. The extraction of the superfluous colour requires from five to fifteen minutes, according to the thickness and character of thesection, and should on no account be allowed to proceed too far; if it does, the peculiar and important staining-effect of the safranin is lost. As first pointed out by Flemming, it has the peculiar property of staining the nucleus and its contents while it may be totally removed from other parts of the cell; in fact, as in the oyster egg, it may be entirely removed from the nucleus and left only in a part of the nucleolus. The methyl green, on the other hand, does not tend to stain the eggs, but rather the spermatozoa and the cells from which they are derived; and it is one of the most astounding facts known to histological

^{*} These are both aniline colours; the first is hard to obtain, except from dealers in dyers' colours.

chemistry, that although both of these dyes, to begin with, are intimately mixed together in the staining-fluid, the different histological elements of the section exert some kind of selective power by which they absorb and hold mainly the This peculiar property of the two colours, one colour only. even when mixed together, enables one to distinctly map out the relations of the sexual elements in the reproductive follicles, the nuclei of the ovarian ova being stained red by the safranin, and the heads of the spermatozoa bluish green by the methyl green. The foregoing is mainly the method to which I have had recourse in working out the sexual characteristics of Ostrea edulis. Simpler staining-methods suffice in the case of Ostrea virginica and Ostrea angulata. A single colour used in staining sections of O. edulis is liable to lead to error, in consequence of the peculiar mode in which the spermatozoa are packed together in oblong clusters, which are often of about the size of the ovarian ova. This egg-like appearance of the masses of unripe spermatozoa in the follicles of the reproductive organs of the common oyster of Europe misled me when examining sections stained only with eosin or carmine. The monochromatic effect produced by one colour only gave no hint as to the real relations of ova and spermatozoa in the follicles until high powers were used with special manipulation of the light.

The characteristics of the reproductive organs of Ostrea edulis, O. virginica, and O. angulata are sufficiently marked to be very precisely described and figured, so as to enable any person to appreciate the differences, especially between the first and the last two. O. edulis is essentially hermaphroditic in the structure of its reproductive organs, while the other two are as distinctly monecious or unisexual. A marked difference is also to be noted in the relative size or calibre of the reproductive follicles in the hermaphroditic and in the unisexual species. In O. edulis the calibre of the generative tubules appears to be relatively much greater than in O. virginica and O. angulata, nor are the tubules so densely crowded together as in the latter species. Up to this time this difference appears to me to be so marked that I think it would be possible to distinguish sections of O. edulis from those of the other two species by means of this one character. other respects the history of the development of the reproductive tissues in both species appears to be similar. all, the sexual tissue arises as a linear interstitial differentiation between the coarse connective-tissue cells of the animal, only that in O. edulis the rudimentary network does not form quite so close a meshwork as in the other two forms here

considered. The tubules have a more extensive anastomosis with each other in the unisexual species than in the hermaphroditic. In all the forms fine vessels pass off from the dorsal and ventral somatic arteries, which tend to branch into vessels of a capillary fineness amongst the reproductive follicles. Thus the glandular portions of the reproductive organs are effectively nourished by supplies of blood passing from the great vessels given off by the heart. These are the principal characteristic features of the reproductive follicles in the hermaphroditic and unisexual forms which are noticed upon comparing the two together. The most important differences between the two forms are to be found, however, in the mode in which the generative elements are produced in each type,

which we will now consider.

In O. edulis the reproductive glands, when well developed, show in many cases a lining of large nearly mature ovules or ovarian eggs at intervals; and insinuated between them large coarsely granular bodies may be observed, in which large irregular nuclear bodies are often imbedded. These nuclear bodies are further distinguished from those of the ovules by their oval or oblong and often irregular form, and by containing a dense mass of granules which absorb safranin in such quantity as to become opaque. This granular chromatin, as it would be designated by Flemming, is usually aggregated at the centre of the nuclear or cellular mass, whichever it may be, and is furthermore apt to conform to a certain extent to the external outline of the body which contains it. From these bodies the rounded granular cells appear to arise which fall into the eavity of the tubule or folliele, there to undergo further segmentation, and finally break up into spermatozoa with spherical heads and filiform tails or flagella. Even (in some cases) where no spermatozoa are as yet revealed by the methyl green, these rounded spermogens or spermatoblasts are to be seen free in the centre of the follicles. Usually, however, the spermatoblasts have been crowded towards the external end of the tubule, where they have undergone differentiation into spermatozoa. The spermatozoa are often on this account so crowded together at the outlet of the tubules, passing even into the superficial duets, that when acted on by the methyl green they are revealed as a dense almost opaque dark bluish-green mass. The ovules, on the other hand, which may be quite nearly mature, remain unstained, except their spherical clear nucleus and nucleolus, which is double, as if formed of two conjoined spherules. If the safranin has been washed out of the nucleus, the one spherule of the nucleolus only is apt to retain the colour. The

peculiar nucleus of the ovules at once distinguishes them from the elements which later break up and become the spermatozoa. Apparently every phase of the spermatogenetic process is under way in the follicles, while more or less nearly mature ovules may be adherent to the walls of the same tubules. In some specimens I find the tubules to contain nothing but ova, with little or no trace of spermatoblasts; in others, again, both classes of products may be present in about the same condition of maturity. In still others little else but spermatozoa are to be found, but, adherent to the walls of the follicles, cells are to be found which have the nucleus so characteristic of the more mature ovules. These, I am inclined to believe, are the representatives of what will later become ova, and not the representatives of spermatoblasts. It is a singular fact that the spermatozoa have a tendency in O. edulis to cling together in masses of about a uniform size. Though the spermatic particles which compose these masses are somewhat separated from each other, if compressed together they would evidently form a body about the size of the spermatoblasts from which they were derived. Later they tend to break up and form a more homogeneous granular mass at the outlet of their parent tubule, where the latter joins the outgoing efferent duct. While it is true that some sections of O. edulis show little evidence of the presence of any thing else but the product of one sex, it appears to me that there is sufficient evidence of the hermaphrodite character of the generative glands of the species presented by a pretty large series of sections taken from about fifty individuals from different localities along the coasts of Wales, Scotland, England, France, Holland, and Germany. Sometimes a portion only of a section will be hermaphroditic, showing that different parts of the generative glands of the same animals may be of different sexes. The result of this arrangement is that it is scarcely possible for the eggs to escape impregnation by the milt generated alongside of them, and we may, I believe, fairly assume that Ostrea edulis is a self-fertilizing hermaphrodite.

The condition of things in the generative tubules of Ostrea virginica and angulata is very different, as may be gathered from the following account. In the first place I have never found any evidence of hermaphroditism either in the living animal or in sections of the reproductive organs. The mode of pressing out the spawn from the gland and ducts of O. virginica, and the physical test used to determine the sex of the products in practical work during the last season, afford the most positive demonstrations of the unisexuality of that species.

Examining sections, however, we never find either in the reproductive follicles of O. virginica or of O. angulata any evidence of the coexistence of ovules and spermatozoa. In fact the mode of spermatogenesis in the unisexual species is very different from that of the hermaphroditie. As indicated in Brooks's figure of a part of a section of a male oyster, the spermatozoa are peculiarly arranged in the follicle or tubule. Upon applying a high power (500 to 800 diameters) I find that the heads of the spermatozoa show a very marked tendency to be arranged in rows like beads, and not in oblong clusters as in the hermaphroditic species. Moreover the walls of the generative tubules are lined by relatively very much smaller spermatoblasts than those found free in the reproductive follicles of the hermaphrodite form. This spermatogenetic layer is often very marked in the males of the unisexual species, and even at an early stage of the functional activity of the testicular organ presents much the same struc-The rows of spermatozoa already ture that it does later. alluded to also have a tendency to be bent towards the outlet of the tubules, giving rise to a fringe-like appearance on either side of the follicle with a clearer space between the edges of the fringe-like masses of spermatozoa. In fact it is plainly to be seen that the spermatozoa are being budded off from the spermatogenetic layer, and that the appearances just described are a result of that process. It results from this that the structural peculiarities of the testicular tubules are very characteristic, so that once recognized they will never afterwards be confounded with the arrangement observed in the ovary of the female, where, as in the hermaphrodite species, the ova may be seen in different stages of development, though, where the majority of the ovules have attained nearly full development, it may happen that few of the nascent ovules closely adherent to the walls of the follicles are visible.

The distinction between Ostrea edulis and the American and Portuguese species is therefore very marked and important. Möbius ('Die Auster und die Austernwirthschaft,' Berlin, 1877, p. 19) says of their species:—"Oysters are hermaphrodites. In the largest number of individuals, in the whole reproductive organ 1 found only spermatozoa, but no eggs. In seven oysters which carried blue brood in the beard, the sexual gland contained only spermatozoa. Three oysters with younger white embryos in the beard had no spermatozoa in the sexual gland. In the most of the brood-bearing oysters the sexual gland contained neither eggs nor spermatozoa. Of 309 oysters which were taken on the 25th of May from four different banks east of the island of Sylt, and afterward examined

from May 26 to June 1, 18 per cent. were hermaphroditic, and of the remaining 82 per cent. one half were egg-bearing, the other half sperm-bearing. In none were the sexual products completely mature. From these observations I conclude that the eggs and spermatozoa do not develop simultaneously but successively in the sexual gland; that spermatozoa may be developed very soon after the discharge of the ova, and that probably one half of the oysters of one locality during a breeding-period produce only eggs and the other half produce only spermatozoa." To the same effect are the statements of Lacaze-Duthiers; but Davaine seems to have first noticed the peculiar aggregations of spermatozoa in oval masses in Ostrea edulis. Brooks thinks "Gerbe's statement, that among the 435 European oysters one year old he found 35 with young, 127 with ripe eggs, and 189 with ripe semen, seems to be sufficient to show the incorrectness of Lacaze-Duthiers's conjecture that the functionally male condition

precedes the functionally female condition."

This is about the state of the controversy at present in regard to the breeding-habits of Ostrea edulis. The only authority, as far as I am aware, who distinctly takes the ground that eggs of this species are fertilized in the reproductive organs is Horst, who says, "Not only do the embryos pass through their first stages of development within the mantle-cavity of the adult, and impregnation occurs internally instead of externally, but it may also be said that the eggs and spermatozoa come into contact in their passage out of the generative glands." It is barely possible, indeed probable, if my memory serves me rightly, that Davaine has put similar observations upon record. Horst also distinctly asserts that the normal development of the embryos of Ostrea edulis cannot take place outside of the parent. M. Berthelot, according to Mr. Brandely, has discovered that the fluids in the mantlecavity of O. edulis contain albumen in a notable proportion, upon which the young are supposed to be nourished. Mr. Brandely has found, by direct experiment, that in the case of O. angulata it is possible to artificially impregnate the eggs. His attempts to fertilize the eggs of O. edulis with the milt of O. angulata and vice versa were unsuccessfully repeated at different times for the last two years. I am now also uncertain in regard to the identity of the species of which Lieut. Winslow succeeded in artificially impregnating the eggs at the mouth of the St. Mary's River, in the Bay of Cadiz, Spain, which he says were natives, the variety having existed and flourished in the bay for as far back as could be remembered. I quote his description of the specimens he used in his experiments as follows:—" In appearance they were quite similar to the American species (Ostrea virginica), having long shells of from 1 to 3 inches in length, rougher and thicker than is usually the case with the European oyster." This remark raises the question whether the experimenter was not really working with O. angulata instead of O. edulis. The locality where he got his specimens and where he conducted his experiments also makes it not improbable that he was in reality working with the native unisexual species, O. angulata.

To return to the question of the breeding-habits of Ostrea edulis, it appears to me that we cannot very well question the authority of Möbius, Lacaze-Duthiers, and Horst, in regard to the bisexual state of the reproductive organs. My investigations also give some countenance to the fact of a preponderance either of eggs or of spermatozoa in different individuals; in fact, in some cases the one or the other seems to be almost exclusively the mature product. But we are not yet in a position to arrive at a conclusion in this matter, because of the scantiness of the observations which have hitherto been made. hypothesis that the spermatozoa are drawn from without into the generative ducts by the ciliary action of the gills and mantle may be dismissed with the remark that microscopic investigation, to my mind, has effectually disposed of the probability of any such a state of affairs. We may see the spermatozoa in course of development in the same follicle with the ova, which is conclusive proof that the milt has not been derived from without, from the water into which it had been discharged by neighbouring individuals. In truth, we find in some cases the spermatozoa present so deep down in the utmost ramifications of the generative follieles that it is not conceivable that they should have been drawn in from without.

As to the alternate activity of the organs in producing ova and spermatozoa, there is a possibility that such is the case, but, as stated at the outset, there is as yet no conclusive proof of the fact. Certain it is that I have yet to see sections of O. edulis in which both ova and spermatozoa are not present in some condition of development at the same time. If the one be not present in a fully developed state, developing traces of it may be discovered; or even a very minute quantity of developed milt or a few developed eggs may be present in some one follicle, while in the others there are perhaps exclusively eggs or exclusively milt in a developed condition. I am aware that this view of the matter is opposed to the current doctrine that nature provides against continuous interbreeding; but when we find the eggs and milt about equally

advanced in development in the same follicle, what is there to prevent self-fertilization; in fact, what else can be the mode

of reproduction?

In some of the sections of O. edulis examined by me, the ovules already measured $\frac{1}{250}$ of an inch in diameter, showing them to be about twice the size of the ripe eggs of O. virginica and O. angulata, in both of which the ova are of about the same size when mature. Estimates which I have made, based on the figures of the eggs of O. edulis given by M. Davaine, show them to be $\frac{1}{130}$ of an inch in diameter. Estimates based on the figures of Lacaze-Duthiers give $\frac{1}{270}$ of an inch, while Möbius and Horst give the size of the young fry at 1 of an inch in diameter. The spherical heads of the spermatozoa of the three species here discussed measure about the same, or approximately $\frac{1}{13000}$ of an inch in diameter. The clusters of spermatozoa of O. edulis measure approximately $\frac{1}{750}$ of an inch in diameter. The spherical unsegmented spermatoblasts which break up into spermatozoa in O. edulis measure 1500 of an inch in diameter. The nucleus of the ovarian eggs of O. edulis measures not quite $\frac{1}{750}$ of an inch in diameter. nucleus of the ovarian egg of O. angulata measures approximately 1333 of an inch in diameter, which is about that of the nucleus of the egg of O. virginica. The large spherule of the nucleolus of the egg of O. edulis measures 1 of an inch in diameter; the small spherale, which is stained red by the safranin, measures $\frac{1}{4250}$ of an inch; the long diameter of the conjoined spherules is $\frac{1}{1750}$ of an inch. The long diameter of the nucleolus of the egg of O. angulata and O. virginica is about $\frac{1}{4000}$ of an inch. A slide in my possession containing some of the brood of O. edulis shows that, even after it has acquired both valves of the shell within the beard of the mother oyster, the brood varies greatly in size. I find, for example, that such fry measures from 110 of an inch down to as small as $\frac{1}{150}$. This brood, like that of the American oyster, has not yet acquired any umbonal prominences at the hinge end of the valves. Before this occurs in the American-oyster embryo considerable growth has taken place; but when the shell already covers the body the whole embryo, contrary to what is found in the European species, measures little, if any, more in diameter than the egg, or about $\frac{1}{500}$ of an inch. Later, when the embryo has grown considerably, and when it is on the eve of attaching itself permanently, it measures from $\frac{1}{80}$ down to $\frac{1}{90}$ of an inch in diameter. The mode of fixation of the fry of both species is probably the same; but the mode of incubation (the one in the mother, the other in the open water), we see, is widely different, differing as greatly in this respect as do the eggs in size and details of construction, as shown by the measurements which I have given. It must not be forgotten, however, that the material from which I prepared my sections was received from Europe in January and March, when it is to be supposed that the reproductive organs were not yet fully developed, and that consequently the dimensions of the ovarian ova as found by me are rather to be considered as being below than above their true ones when fully developed at the height of the

spawning-season.

It is a very remarkable fact that one finds individual specimens of oysters in which the reproductive organs have undergone total atrophy or wasting-away at the completion of the spawning-season. Examining sections through the body-mass of spawn-spent oysters taken from their native waters in August last, I find that the whole of the connective tissue subjacent to the mantle, and between the latter and the liver, especially over the sides of the body-mass, has disappeared, together with all traces of the reproductive organs, including the superficial branches of the efferent duets. At the first bend of the intestine there is still some of the connective tissue remaining; but even here and in the mantle it has changed its character entirely, and become very spongy and areolar, instead of solid and composed of large vesicular cells such as are met with when the animal is in a better condition of flesh. In fact, it appears as if this mesenchymal or connective-tissue substance had been used up and converted into reproductive bodies (generative products) in the ease of the spawn-spent and extremely emaciated individuals. In sections of individuals in various conditions from that in which the rudimentary network of generative tubules has just appeared in the connective tissue, on up to those in which the reproductive tissues are enormously developed in bulk and proportion to the mass of the remaining structures, there is a perfect gradation from their complete absence to their full development. would appear to be very strong evidence in support of the theory that the reproductive follieles, or tubules, are developed anew each season directly from the specialization of certain strings or strands of connective-tissue cells.

Many animals manifest a periodic development of the glandular portions of the reproductive organs; but I know of no form in which there is any such presumptive evidence that these organs are annually regenerated and finally altogether aborted as seems to be the case with the oyster. Together with the changes here described, the most remarkable changes in the solidity and consistence of the animal take place. The

shrinkage of a spawn-spent oyster in alcohol or chromic-acid solution is excessive, and will, when complete, reduce the animal to one tenth of its bulk while alive. This shrinkage is due to abstraction of the water with which the loose spongy tissue of the exhausted animal is distended. A so-called "fat" oyster, on the other hand, will suffer no such excessive diminution in bulk when placed in alcohol or other hardening fluid. In consequence of this variable development of the reproductive organs as well as that of the connective tissue of the body-mass, the amount of solid protoplasmic material contained in the same animal at different times under different conditions must vary between wide limits. And inasmuch as the nutritive and reproductive functions of animals are notoriously interdependent, it follows, in consequence of the enormous fertility of the oyster, that a vast amount of stored material in the shape of connective tissue must be annually converted into germs and annually replaced by nutritive processes. Plentitude or dearth of food are also to be considered: but it now becomes a little easier to understand the physiological interdependence of the reproductive function and the so-called fattening process.

To a great extent what has been remarked in the preceding paragraphs of the wasting-away of the reproductive organs in Ostrea virginica seems to apply also to O. edulis and O. angulata. The last species has an extraordinarily thick bodymass, with the stratum of reproductive follicles of remarkable thickness, averaging a much greater development than I have ever seen in any other form. When the contents of this great mass of tubules has been discharged a diminution in the bulk of the body-mass must naturally ensue, probably accompanied by a wasting-away of the connective tissue and tubules, such as apparently occurs in the American species. From what I have seen of the generative tubules of O. edulis in sections, they are evidently regenerated much as in O. virginica. In a few specimens I find them almost entirely gone, or present

only in an extremely rudimentary state.

VI.—Occurrence of Rhinodon typicus, Smith, on the West Coast of Ceylon. By A. HALY.

On January 5th a large female shark which I identify as *Rhinodon typicus* was entangled in the nets at a fishing-village called Moratuwa, twelve miles south of Colombo. The native population were greatly excited, and flocked in large